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# Health Effects of Fine- and Coarse-Mode Particulate Matter: Exposures by Inhalation and Intratracheal Instillation

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Because coarse mode particles are rarely studied in their existing size ranges (greatest mass about 5-7 um, aerodynamic diameter), we investigate the effects of four such particles, quartz, ferric oxide, calcium carbonate, and sodium feldspar, on host defenses against bacterial pulmonary infection. Mice which received intratracheal instillations of 10, 33, and 100 ug/mouse were exposed within an hour to aerosols of viable Streptococcus, and pneumonia-induced mortality was measured. At 33 and 100 ug/mouse, all particles significantly increased mortality. At the lower dose, only Fe2O3 caused a significantly increase in mortality. To evaluate potential delayed effects, mice were challenged with the bacteria 24 h after exposure to 100 ug particles/mouse. Delaying the challenge did not significantly alter the response, except for the sodium feldspar group for which a partial recovery was observed.

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# EPA ENVIRONMENTAL RESEARCH BRIEF

## Health Effects of Fine- and Coarse-Mode Particulate Matter: Exposures by Inhalation and Intratracheal Instillation

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### Abstract

The effects of fine- and coarse-mode particles on the respiratory defense system were studied in a two-phase investigation. Aerosols of fine-mode particles were used in inhalation exposures, whereas coarse mode particles were administered by intratracheal instillation.

Rangefinding acute and repeated exposures to ammonium sulfate or ammonium nitrate aerosols were followed by subchronic exposures to ammonium sulfate, sulfur dioxide and ozone mixtures to elucidate the potential impact on health of interactions of these particles and gases. The effects of exposure to 0.2 mg/m$^3$ ozone, or to a mixture containing ozone, 13.2 mg/m$^3$ sulfur dioxide and 1.04 mg/m$^3$ ammonium sulfate for 5 hr/day, 5 days/week for up to 103 days were evaluated in mice in a series of *in vivo* and *in vitro* health effects assays following the exposures. Statistically significant changes were observed in susceptibility to streptococcal pneumonia, in the bactericidal activity of alveolar macrophages, in *in vitro* cytostasis in tumor target cells cocultured with peritoneal macrophages and in splenic T-lymphocyte function measured by blastogenesis to mitogens and alloantigens.

The effects of coarse-mode particles of quartz, ferric oxide, calcium carbonate and sodium feldspar, on host defenses against pulmonary bacterial infection were also investigated. Mice receiving intratracheal instillations of 10, 33 or 100 μg/mouse were challenged with *Streptococcus* sp. aerosols within 1 or 24 hr after the exposure. Mortality from streptococcal pneumonia was significantly increased by exposure to all particles at the 33 or 100 μg/mouse concentration. Delaying the infectious challenge to 24 hr caused partial recovery in mice exposed to sodium feldspar but not to those exposed to the other particles.

The following publications resulted from the studies: Effects of Subchronic Exposure to a Mixture of $O_3$, $SO_2$, and $(NH_4)_2SO_4$ on Host Defenses of Mice. Catherine Aranyi, Stanley C. Vana, Peter T. Thomas, Jeannie N. Bradof, James D. Fenters, Judith A. Graham, and Frederick J. Miller; J. of Toxic. Environ. Health 12:55-71, 1983.

The Effects of Intratracheally Administered Coarse Mode Particles on Respiratory Tract Infection in Mice. Catherine Aranyi, Jean L. Graf, William J. O'Shea, Judith A. Graham, and Frederick J. Miller; Toxicol. Lett. 19:63-72, 1983.

### Introduction and Summary Text

Atmospheric aerosols are classified as fine- and coarse-mode according to particle size. Sulfate, nitrate, and ammonium salts and organic compounds are generally found as fine-mode particles produced by condensation, growth and agglomeration. Coarse-mode particles originate from mechanical abrasion of earth crustal materials and typically contain iron, aluminum, titanium, silica and calcium. The demarcation between fine- and coarse-mode urban aerosols is at approximately 2.5 μm aerodynamic diameter with the greatest mass of particles occurring at 0.4 to 0.5 and 5 to 7 μm for fine- and coarse-mode particles respectively. Although a significant amount of health effects information is available, many uncertainties exist, particularly with respect to effects on host defenses against pulmonary infection of these particles. Epidemiological

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studies have shown associations between exposure to mixtures of sulfur dioxide ($SO_2$) and particles and increased incidence of pulmonary infections. However, little information is available to define the chemical species of the particles which were responsible for these observations.

Initial studies were conducted using single and/or repeated exposures to ammonium sulfate [$(NH_4)_2SO_4$] and ammonium nitrate ($NH_4NO_3$) to obtain rangefinding data which form the basis for the design for the subchronic study. The purpose of the subchronic study was to evaluate the influence of $SO_2$ and particles in the presence of ozone ($O_3$) on essential host defense functions. Sulfur dioxide $(NH_4)_2SO_4$, and $O_3$ were chosen because of (a) our interest in $SO_2$-particulate interactions; (b) because $(NH_4)_2SO_4$ is a major component of fine-mode urban aerosols; and (c) because of the ubiquitous presence of $O_3$ and its effects on host defenses against infection. The 5-hr experimental exposure period corresponds to the ambient $O_3$ peak patterns usually observed in urban environments. The particle-size distribution of $(NH_4)_2SO_4$ was chosen to correspond to that of this pollutant in fine-mode urban aerosols. Since $SO_2$ and $(NH_4)_2SO_4$ given singly at the same or at higher concentrations had no effect on the major endpoints to be examined, (Ehrlich 1979; Ehrlich et al. 1978) there was an insufficient rationale for further study of these pollutants alone. Thus, a mixture of $O_3$, $SO_2$ and $(NH_4)_2SO_4$ aerosol, was decided upon, especially since $O_3$ co-exists with ambient aerosols. To evaluate the contribution of $O_3$ to any effects observed with the mixture, an $O_3$ group was included. The control group was exposed to filtered air.

Several health effect parameters were assessed to evaluate effects of the exposures. Changes in susceptibility to respiratory infection in which experimental and control mice are simultaneously challenged with streptococcal aerosol and mortality rates are compared, was selected because of the sensitivity of this model in determining the impact of pollutants on several host defense mechanisms. Pulmonary bactericidal activity was measured to determine if the exposures affected the function of alveolar macrophages *in situ*. The activity of alveolar macrophages and other free lung cells obtained by tracheobronchial lavage was also examined. *In vitro* peritoneal macrophage cytostatis to tumor target cells was measured to assess whether the inhalation exposures affected extrapulmonary macrophage activation, a potential defensive capacity against tumor growth. The effect of the exposures on the systemic immune system was studied whereby the blastogenic response of splenic lymphocytes to mitogens and alloantigens, and plaque-forming cell response to sheep red blood cells were used to measure the cell-mediated and humoral immunity, respectively. Tracheal ciliary beating frequencies and alterations in cytology in the tracheal epithelium from exposed animals were assessed.

Intratracheal instillation was used for exposures to various coarse-mode particles to obtain a toxicity ranking that would provide guidance for future inhalation studies. The use of this exposure method circumvented the extreme difficulty in generating stable coarse-mode polydisperse aerosols at sufficient mass concentrations and for adequate length of time. Iron oxide, calcium carbonate and kaolin (clay) were chosen for evaluation, as relatively common coarse-mode particles in the atmosphere. Silicon doxide was included since silica in fine-mode form is known to be toxic and it is found in ambient aerosols in the coarse-mode. In selecting the actual compounds to be used, the primary consideration was to obtain materials as close as possible in crystal structure, crystal habit, and phase purity to those encountered in ambient atmospheric aerosols. Therefore, hematite ($Fe_2O_3$) was selected as the typical iron oxide compound, calcite ($CaCO_3$) as the calcium carbonate, and quartz ($SiO_2$) was selected as the free silica compound. Because coarse-mode clay particles in the atmosphere are actually agglomerates and would therefore disperse when suspended in the saline required for intratracheal instillation, its geological precursor, the sodium feldspar albite ($NaAlSi_3O_8$), was used.

## 1. Fine-Mode Particles

### *a. Acute and repeated exposures to ammonium sulfate and ammonium nitrate aerosols*

Single 3-hr exposures to approximately 2.5 mg/m$^3$ $(NH_4)_2SO_4$ aerosol did not affect susceptibility to respiratory infection and pulmonary bactericidal activity in mice and did not produce any significant alterations intracheal explants in mice and hamsters. Similarly, only minor changes were noted in the various health effects parameters examined after 5, 10 or 20 daily 3-hr inhalation exposures to approximately 1 mg/m$^3$ $(NH_4)_2SO_4$ aerosol (59% of the particles had $\leq$1.0 $\mu$m MMAD). After 5 daily exposures to the aerosol, ATP levels of pulmonary free cells were decreased in both female and male mice, but no consistent changes in these parameters were observed after 10 or 20 daily exposures. Altered tracheal epithelial cytology was noted only in hamsters after 5 exposures. Goblet cell hyperplasia was observed in mouse tracheas after 10 and 20 exposures. Slight alterations of alveolar shape and wall thickness were observed by scanning electron microscopy after all three exposure regimens. No changes were seen in susceptibility to respiratory infection, pulmonary bactericidal activity, type or viability of free pulmonary cell, tracheal ciliary beating frequency or pulmonary histology.

The effects of a single 3 hr exposure to 2 mg/m$^3$ of $NH_4NO_3$ and 5 and 20 3-hr exposures to 1 mg/m$^3$ $NH_4NO_3$ aerosol (MMAD = 0.9 $\mu$m, $\sigma$g = 1.8) were evaluated in mice or hamsters. In general, only a few minor changes were found in the health effects parameters examined. After the single exposure, a decrease in tracheal cilia beating frequency, reduced percent of normal tracheal epithelium, and minor histologic changes in tracheal epithelium were seen in hamsters. After 5 exposures, significant decreases in cilia beating frequency and percent of normal epithelium were seen only in the hamster tracheal organ culture. After 20 exposures, slight congestion and enlarged alveolar pores were noted in the lungs of mice. Total pulmonary free cell counts tended to be greater than control value after 1 or 5 exposures, but lower than control values after 20 exposures and the ATP levels were lower in pulmonary free cells lavaged from exposed mice. No changes were seen at any time in susceptibility to respiratory streptococcus infection, in pulmonary bactericidal activity and in differential counts or viability of pulmonary free cells.

### *b. Subchronic exposure to a mixture of ozone, sulfur dioxide and ammonium sulfate*

Mice exposed 5 hr/day, 5 days/week for up to 103 days, to 0.2 mg/m³ $O_3$ or to a mixture of 0.2 mg/m³ of $O_3$, 13.2 mg/m³ $SO_2$ and 1.04 mg/m³ $(NH_4)_2SO_4$ showed significant increase in susceptibility to streptococcal infection when compared to filtered air controls. Bactericidal activity was significantly enhanced in the lungs of mice exposed to the mixture as compared to those exposed to filtered air or to $O_3$ alone. The total number and distribution of the free cells lavaged from the lungs, as well as cellular ATP levels were not affected. At a target-to-effector-cell ratio of 1:10, *in vitro* cytostasis in tumor target cells cocultured with peritoneal macrophages from the exposed mice was significantly enhanced by exposure to $O_3$ or the pollutant mixture group relative to controls. The magnitude of the effect of the mixture was greater than that of $O_3$ alone. No such effects were observed when the target-to-effector cell ratio was 1:20. Splenic T-lymphocyte function, as measured by blastogenesis to mitogens and alloantigens, was decreased by exposure to $O_3$ alone and increased by exposure to the mixture. Splenic B-cell function and macrophage antigen processing, as measured by the generation of antibody plaque-forming cells, was not affected.

## 2. Coarse-Mode Particles

The effects of coarse-mode quartz, ferric oxide, calcium carbonate, and sodium feldspar particles on host defenses against bacterial pulmonary infection were investigated. Intratracheal instillations of 33 and 100 μg/mouse, resulted in significantly increased mortality from streptococcal pneumonia. At 10 μg/mouse, only ferric oxide caused a significant increase. To evaluate potential delayed effects, mice were challenged with the bacterial aerosol 24 hr after instillation of 100 μg/mouse. Delay of the challenge did not significantly alter the response, except for the sodium feldspar where a partial recovery was observed. Intratracheal instillation of 100 μg/mouse had no significant effect on pulmonary bactericidal activity. For the model system used, it appears that ferric oxide, calcium carbonate and sodium feldspar have effects roughly equivalent to quartz.

## Conclusions

During subchronic exposures to mixtures consisting of $O_3$, $SO_2$ and $(NH_4)_2SO_4$ it appeared that the presence of 0.2 mg/m³ of $O_3$ was the significant cause of the increase in susceptibility to streptococcal infection.

Total and differential cell counts, viability, and ATP levels in the free cells lavaged from lungs of mice exposed to the pollutants did not differ significantly from those in control mice exposed to filtered air. This is in agreement with results of 20 3-hr exposures to 1 mg/m³ $(NH_4)_2SO_4$ and with studies of Ehrlich (1980), showing that exposure to similar concentrations of $SO_2$ for up to 3 months did not change the phagocytic activity of macrophages lavaged from the lung.

Pulmonary bactericidal activity was significantly higher in the mixture-exposed group compared to the air controls or to the $O_3$-exposed group. This increase might be due to activation of the alveolar macrophages after having phagocytized the inhaled particles. In previous studies (Aranyi, 1981; Aranyi et al., 1981), increased bactericidal activity was observed in lungs of mice exposed to aerosols that did not have any significant effects on host defenses; inhalation of more toxic particles appeared to overcome this initial increase, and depressed the bactericidal capacity of the macrophages.

The increased *in vitro* cytostasis to tumor target cells observed for peritoneal macrophages lavaged from the exposed mice, indicate a possible activation of these macrophages by the exposures. The pollutant mixture had a significantly greater effect than $O_3$ alone. The studies demonstrated that exposure to $O_3$ alone or to the mixture altered splenic T-lymphocyte function as measured by blastogenesis to mitogens and alloantigens. The extent of the change was related to the pollutants treatment. Splenic B-cell and T-helper cell function and macrophage antigen processing, as measured by the generation of antibody plaque-forming cells, was not affected by exposures. Thus, these exposures caused extrapulmonary effects, hitherto unrecognized.

Intratracheal instillation was used to examine specific coarse-mode particles common in the atmosphere. The studies demonstrated that intratracheal exposure to $Fe_2O_3$, $CaCO_3$, $SiO_2$ and sodium feldspar particles of 5 to 7 μm MMAD increased streptococcal-induced mortality in mice. This mortality has been correlated primarily, but not solely, to alveolar macrophage function, suggesting that the presence of the particles depressed alveolar macrophage function. Such depression was expected following exposure to $SiO_2$ (Allison et al., 1979) and possibly sodium feldspar, insofar as it is similar to kaolinite (White and Kuhn, 1980). However, bactericidal activity, which is a more direct measurement of alveolar macrophage function than the infectivity model was not affected. Thus it appears that decrements in host defense mechanisms (in addition to alveolar macrophages) such as the mucociliary escalator and edema, may have been also involved. The approximate equivalency of the effects of silica to the other coarse-mode particles indicates that coarse-mode particles cannot be assumed to be nontoxic.

## Recommendations

Results of subchronic inhalation studies indicate that exposure to 0.2 mg/m³ (0.1 ppm) $O_3$ causes alterations in some pulmonary defense systems. For some parameters, exposure to mixture containing $O_3$, $SO_2$ and $(NH_4)_2SO_4$ was more effective than exposure to $O_3$ alone. Given the known low toxicity of $SO_2$ and $(NH_4)_2SO_4$ for host defense mechanisms, their role in admixture with $O_3$ should be further investigated. Since humans are usually exposed to complex mixtures of pollutants it is important to elucidate the causative agents in the mixture or to define a synergistic/antagonistic relationship.

The immunological studies indicated that the mixture and $O_3$ caused effects to the spleen and cells in the peritoneum. Examination of such extrapulmonary effects is rare, requiring more research to enable improved understanding of the full array of effects of these pollutants. In addition,

since the systemic immune response was affected, it would be of interest to investigate the pulmonary immune response since this system would receive more direct exposure to higher doses.

Intratracheal instillation of coarse-mode particles of $SiO_2$, $Fe_2O_3$, $CaCO_3$ and sodium feldspar, caused decreased resistance to respiratory infection. Therefore it is important to confirm and characterize these responses further using inhalation exposures.

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